

FULL PAPER

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Phyllactinia chubutiana: a new species of Erysiphales from Patagonia (Argentina)

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Abstract *Phyllactinia chubutiana*, a parasite on leaves of *Lycium chilense* (Solanaceae) collected in the arid Patagonian steppe, is proposed as a new species. The new combination *Ovulariopsis insolita* is introduced for its anamorph previously described as *Oidium insolitum*.

Key words Erysiphaceae · Molecular phylogeny · *Oidium* · *Ovulariopsis* · Powdery mildew

Introduction

In Argentina, two previous records of powdery mildew on *Lycium* species were based only on anamorphic states. Spegazzini (1898) cited a powdery mildew on *L. cestroides* Schldtl. as *Oidium erysiphoides* Fr. Braun et al. (2000) recorded a powdery mildew anamorph on *L. chilense* Miers ex Bertero and described it as *Oidium insolitum* U. Braun, Kiehr & Delhey. Recently, an anamorph agreeing with *Oidium insolitum*, along with a teleomorph belonging in *Phyllactinia* Lév., has been found on *L. chilense* in Patagonia. The taxonomy and phylogeny of this fungus has been proven by means of morphological as well as molecular sequence analyses.

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Materials and methods

Sample sources

The collection of the diseased *Lycium* leaves was performed in the Patagonian Phytogeographical Province characterized by arid shrub-steppe dominated by tussock grasses and shrubs (Roig 1998). The average precipitation is 200 mm/year (Conti 1998). All the collections were deposited in the institutional herbarium of Centro Regional Universitario Bariloche, San Carlos de Bariloche, Argentina, under the acronym BCRU.

Morphological study

The morphological characteristics were examined from fresh and dried materials. The Lactofuchsin boiling method (Shin 2000) was used to restore shriveled structures in dried samples. For observation of haustoria, mildewed leaves with mycelium, conidiophores, and ascomata were soaked in a few drops of lacto phenol cotton blue. Glass slides with fixed material were maintained in a petri dish for 6 weeks. Transverse sections of leaves were made with a razor blade, and selected samples were transferred to glass slides and soaked in fresh lacto phenol cotton blue during 3–4 days to allow the dye to penetrate the tissues.

Observations and drawings were made under 400× and 1000× magnification of a light microscope provided with a micrometer and drawing camera.

Molecular phylogenetic study

Isolation of whole-cell DNA was performed using the Chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The 5'-end of the 28S ribosomal DNA (rDNA) including the domains D1 and D2 and the internal transcribed spacer (ITS) region including the 5.8S rDNA were amplified by polymerase chain reaction (PCR) and then sequenced using direct sequencing as described in Matsuda and Takamatsu (2003).

For molecular phylogenetic analysis, DNA sequences were initially aligned using the Clustal X package (Thompson et al. 1997). The alignment was then improved visually with a word processing program with color-coded nucleotides. Phylogenetic trees were obtained from the data by the maximum-parsimony (MP) method using the heuristic search option of PAUP* 4.0b8 (Swofford 2001). This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Transversions and transitions were treated as equal weight. All sites were treated as unordered, with gaps treated as missing data. The branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero-length branches were collapsed. The strength of the internal branches from the resulting trees was tested by bootstrap analysis (Felsenstein 1985) using 1000 replications.

Results and discussion

Taxonomy of the fungus

Phyllactinia chubutiana Havryl., S. Takam. & U. Braun, sp. nov.

Anamorph: *Ovulariopsis insolita* (U. Braun, Kiehr & Delhey) Havryl., S. Takam. & U. Braun, comb. nov.

Bas.: *Oidium insolitum* U. Braun, Kiehr & Delhey, Sydowia 53(1): 35 (2000). Figs. 1–3

Chasmothecia (ascomata) gregaria, 120–250 µm diametro. Cellulae peridii 11–29 µm diametro. Appendices 8–12, diametri ascomatis (0.5–)1–2 plo longiores, aciculares, hyalinae, basi inflatae. Cellulae penicillatae 16 × 32 µm, ramis ultimis brevis. Asci 15–22, pedicellati, 66–80(–162) × 28–36 µm, 2-spori. Ascospores ovoideae vel ellipsoideae, 26–36 × 15–21 µm.

Holotypus: BCRU 4634, Argentina. On *Lycium chilense*, Provincia del Chubut: Dep. Languiñeo. Piedra Parada. Leg. M. Havrylenko, April 4, 2005.

Etymology: *chubutiana* from Chubut Province from Argentina.

Anamorph: Ectophytic and endophytic mycelium on leaves, amphigenous, white, dense, effuse, persistent, covering the leaf surface. Vegetative hyphae, hyaline septate, flexuous, branched, smooth, 2.5–8 µm wide, some hyphae with dilatated cells constricted at the septa. Appressoria well-developed, nipple-shaped, rod-shaped to bifurcate or irregularly branched, solitary or opposite in pairs. Mycelium entering the leaf tissue through stomatal openings, mainly reaching the mesophyll palisade cells, less frequently the mesophyll spongy cells. Haustoria in epidermal cell not observed. Infecting hyphae producing delicate haustorial filaments that penetrate mesophyll cells and form a globose convoluted structure, 4–6 µm diameter. At the point of the cell-wall penetration, the haustorium is constricted and a collar can be observed. Conidiophores emerge from superficial mycelium near or away from stomata, solitary,

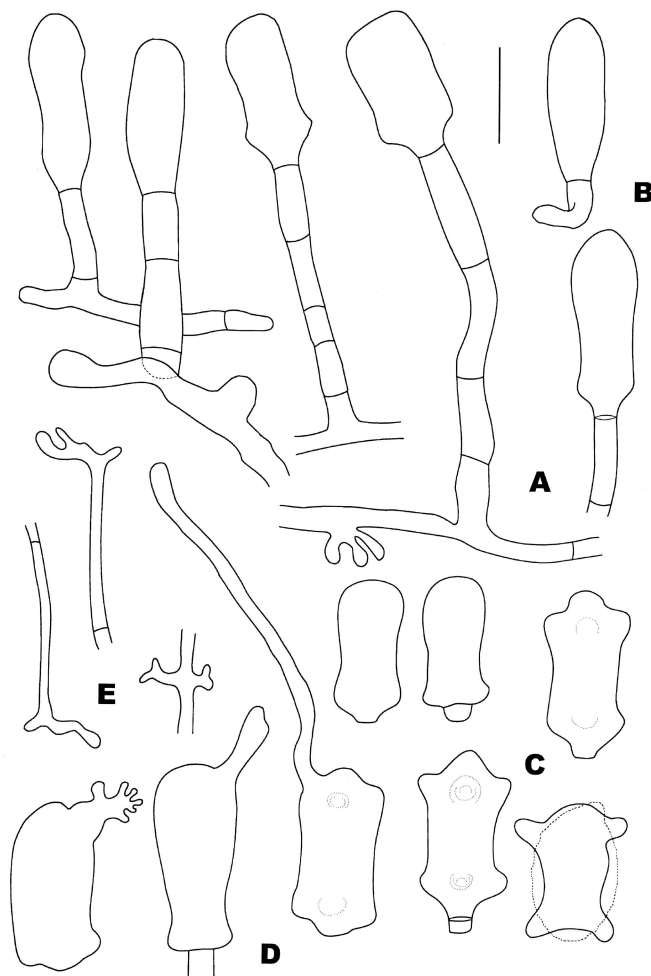


Fig. 1. Anamorph of *Phyllactinia chubutiana*. A Conidiophores. B Young conidia with upper cell attached. C Mature conidia. D Germinated conidia. E Ectophytic hyphae with appressoria. Bar 20 µm

erect, cylindrical, single on hyphal cell, arising from the upper part of mother cells, position central to excentric. Foot cell cylindrical, 16–49 × 5–15 µm, followed by (0–)1–3 equal or shorter cells. Some of the conidiophores do not have foot cells and conidia arise directly from the supporting hyphae. Basal septum always away from the branching point of the mycelium. Conidia solitary, not papillate at the apex. There are two morphological types of conidia. One type, assumed to represent young conidia, is characterized by having ellipsoid to lanceolate conidia. The other type of conidia, considered to be mature, is cylindrical, 27–52 × 12–22 µm, with conspicuous subapical and subterminal protuberances, 4 to 8 in number. Some mature conidia slightly constricted centrally, but most of them are quadrate to rectangular in outline. Two types of subterminal conidial germ tubes were observed: large tubes with a slightly enlarged, simple end and short tubes terminating in a wide multilobed apressorium.

Teleomorph: ascomata mostly gregarious, 120–250 µm in diameter, blackish-brown, globose depressed. Peridial cells polygonal to subrounded, 11–29 µm wide. Appendages 8–10

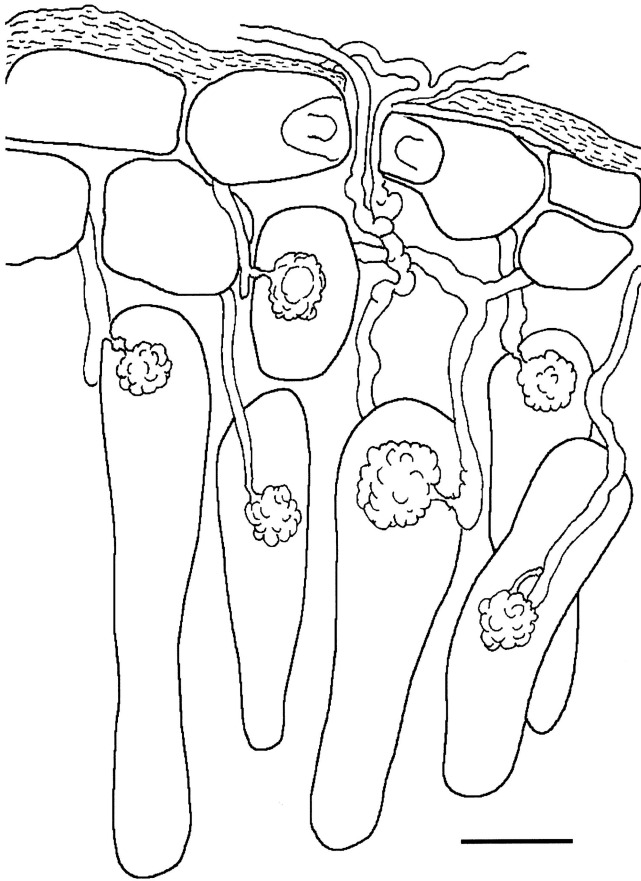


Fig. 2. Haustoria of *Phyllactinia chubutiana*. Infected host plant leaf cross section shows infecting hyphae passing stomatal opening and haustoria in mesophyll cells. Bar 20 μm

in number, arising from the equatorial zone of the ascoma, (0.5)1–2 times as long as the ascomatal diameter, hyaline, simple, acicular, with a bulbous base, 22–40 μm diameter, apex mostly pointed, but occasionally with somewhat curved tip. Penicillate cells: crowded on the upper part of ascoma, stems 16 \times 32 μm , divided at the apex in several very short branches, filaments 1–2 times longer than the stem. Asci: 15 to 22 per ascoma, stalked, thick-walled, 66–80(–162) \times 28–36 μm . Ascospores: two per ascus, ovoid to ellipsoid, with dense cytoplasm containing guttulae, pale gray, 26–36 \times 15–21 μm .

Host: *Lycium chilense* Miers ex Bertero (Solanaceae). Native bush that grows in arid areas of Argentina (Rosow 1999).

Material studied: Argentina. Provincia del Chubut: Dep. Languiño. Provincial route 12, near rural school N 86, Piedra Parada, at 1.5 km from Chubut River. Leg. M. Havrylenko, April 21, 2005, on *Lycium chilense*, BCRU 4634–4636.

Notes: The protuberated conidia and the structure of conidiophores of *Ph. chubutiana* are very unusual and characteristic compared with most other *Phyllactinia* species with clavate conidia. Havrylenko (1995) reported a new *Phyllactinia* species, *Ph. adesmiae* Havryl., with an unusual

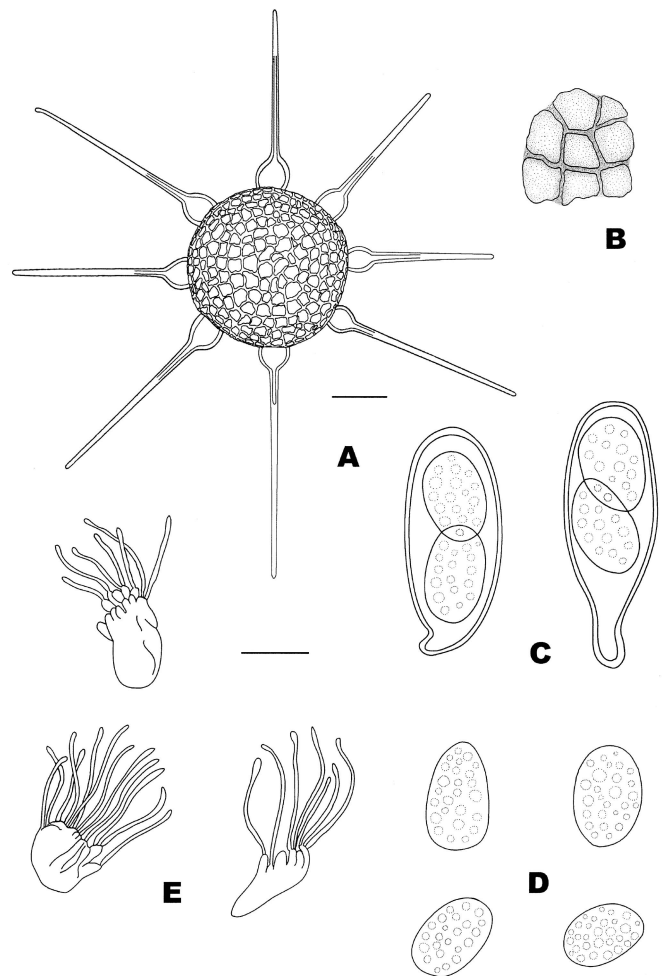


Fig. 3. Teleomorph of *Phyllactinia chubutiana*. **A** Ascoma. **B** Peridial cells. **C** Asci. **D** Ascospores. **E** Penicillate cells. Bars **A** 50 μm ; **B–E** 20 μm

shape of conidia. However, *Ph. chubutiana* is distinguishable from *Ph. adesmiae* by its conidia with a cingulum-like basal ring. Braun et al. (2002) described the shape of *Oidium insolitum* conidia as dumbbell like and compared the unique conidial features of this species with those of *Leveillula lanuginosa* (Fuckel) Golovin and *L. saxaouli* (Sorokin) Golovin. Based on relatively short, narrow conidiophores, conidia formed singly, and irregularly shaped appressoria, they placed this fungus on *Lycium chilense* into the genus *Oidium* Link because the conidiophores in *Phyllactinia* are usually long and narrow. Internal mycelium was not observed. A detailed examination of the mycelium in the new collection from 2005 showed, however, that external as well as internal mycelium is developed. Internal hyphae in combination with a teleomorph belonging in *Phyllactinia* suggest that *Oidium insolitum* has to be reallocated to *Ovulariopsis* Pat. & Hariot, although the short conidiophores and the conidial shape are very unusual for the latter genus.

The present observation indicates that *Ph. chubutiana* has two types of conidia (conidial dimorphism). Although it

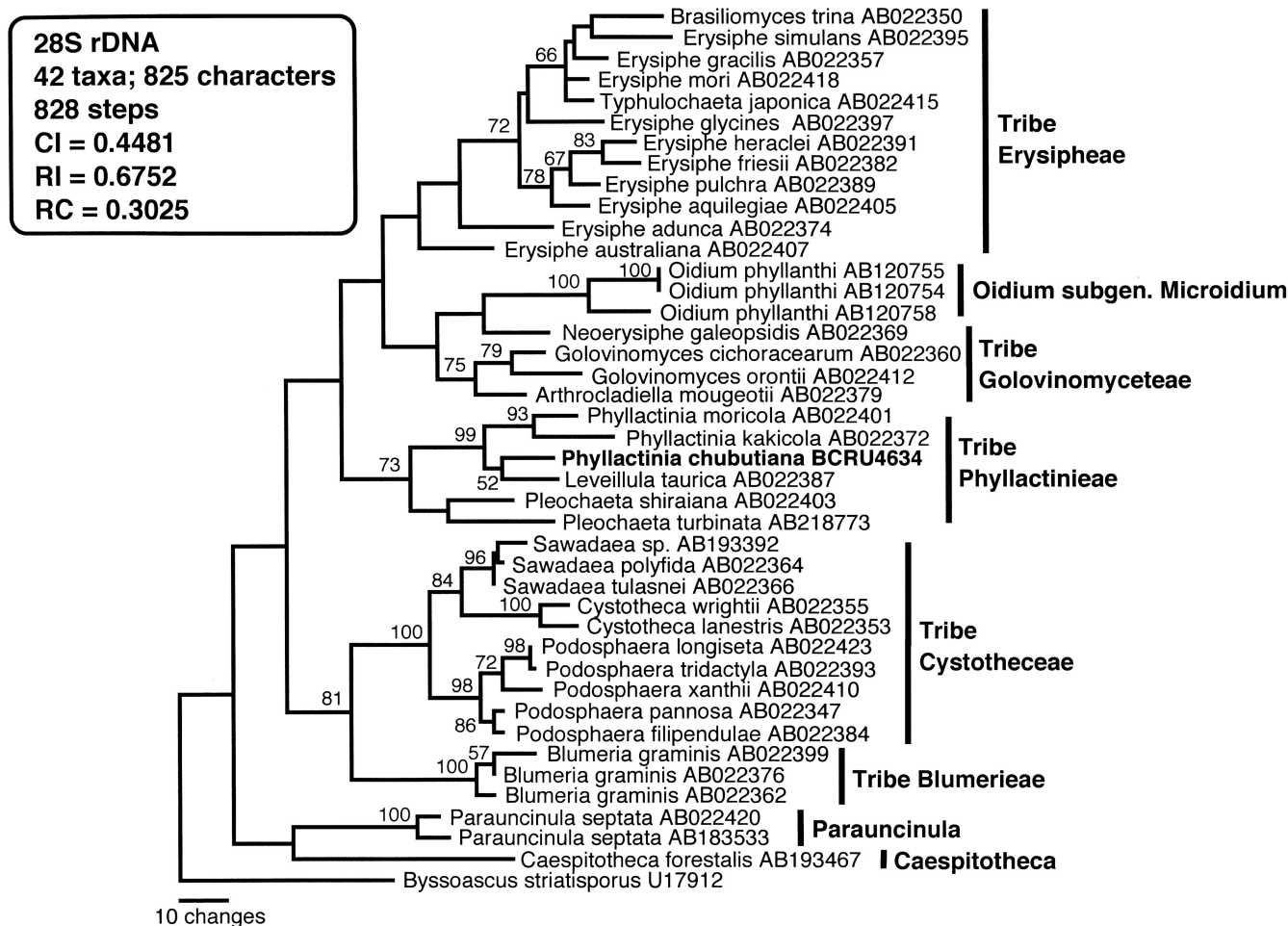


Fig. 4. Phylogenetic analysis based on the 28S rDNA sequences for *Phyllactinia chubutiana* and 40 taxa of the Erysiphales covering all known tribes, and an outgroup taxon. The tree is a single most parsimonious tree with 828 steps, which was found using an heuristic search employing 100 times random stepwise addition option of PAUP* that

treated gaps as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. The bootstrap values of 1000 replications are shown on the respective branch. The respective groups of the Erysiphales are shown in the right of the tree

was not clear whether the two types of conidia correspond to primary and secondary conidia, this result suggests that there are *Phyllactinia* species having dimorphic conidia in their anamorph. Liberato et al. (2004) also reported similar results. They emended the definition of *Streptopodium* R.Y. Zheng & G.Q. Chen that has been regarded as the anamorph of *Pleochaeta* Sacc. & Speg., and accommodated the species having semiendophytic parasitism and dimorphic conidia into *Streptopodium*. However, formation of dimorphic conidia may be a general and common phenomenon in the Erysiphales. In a wide range of powdery mildew fungi, the first conidia (primary conidia) are often distinct in having a rounded apex and being more ovoid, whereas the following conidia (secondary conidia) are apically truncate or subtruncate, being more cylindrical-doliiform. For instance, Limkaisang et al. (2005) recently reported conidial dimorphism in *Oidium heveae* B.A. Steinm. Conidial dimorphism is possibly overestimated to delimit *Streptopodium*. There are many transitional cases between *Ovulariopsis* and *Streptopodium*, and the limits between the two

genera are vague. More comprehensive analyses are required to find morphological characters to distinguish between *Ovulariopsis* and *Streptopodium*. Thus, we tentatively prefer to accommodate the anamorph of *Ph. chubutiana* in *Ovulariopsis*.

The chasmothecia are barely distinct from those of *Phyllactinia guttata* (Wallr.: Fr.) Lév., which is, however, not unusual in *Phyllactinia*. However, the new species is easily distinguishable from all other species of this genus by its unusually shaped conidia.

Molecular phylogenetic analysis

We extracted whole-cell DNA from ascomata of *Phyllactinia chubutiana* isolate BCRU 4634 and determined a total of 1387 nucleotides consisting of the 3'-end of the 18S rDNA, 5'-end of the 28S rDNA including the domains D1 and D2, 5.8S rDNA, and ITS regions. The sequence was deposited in DDBJ under the accession number of

AB243690. Of these, 717 nucleotides of the 28S rDNA were used for the following molecular phylogenetic analysis. Nucleotide sequences of the domains D1 and D2 of 28S rDNA from 40 taxa covering all five tribes of the Erysiphaceae and *Oidium* subgenus *Microidium* (To-anun et al. 2005) were retrieved from the DNA database and used for the phylogenetic analyses. The alignment data matrix consists of 42 taxa and 825 characters, in which 256 sites were variable and 174 sites were phylogenetically informative for parsimony analysis. *Byssoascus striatisporus* (G.L. Barron & C. Booth) Arx (U17912) was used as an outgroup taxon based on Mori et al. (2000a,b). The alignment was deposited in TreeBASE (<http://www.treebase.org/>) under the study number of S1483 and matrix number of M2667.

Twelve equally most parsimonious trees with 828 steps were generated by the MP analysis that differed only in minor branching orders of terminal taxa. A tree having the highest likelihood score among the 12 trees is shown in Fig. 4. The five tribes, viz. the Erysipheae, Phyllactinieae, Cystothecae, and Blumerieae, recognized in the Erysiphaceae (Cook et al. 1997; Braun 1999; Braun and Takamatsu 2000; Mori et al. 2000a) are again supported as respective monophyletic groups. *Oidium* subgenus *Microidium*, proposed by To-anun et al. (2005) as a new subgenus of the anamorphic powdery mildew genus *Oidium*, forms an independent clade, clearly separated from the other five tribes. Recently proposed genera, *Parauncinula* and *Caespitotheca* (Takamatsu et al. 2005a,b), are also supported to be placed in the basal position of the Erysiphaceae. *Phyllactinia chubutiana* groups with *Phyllactinia* and *Leveillula* with 99% bootstrap support in the clade of tribe Phyllactinieae. Sequence similarity of *Ph. chubutiana* is 95.9% with *Ph. moricola* (Henn.) Homma, 95.1% with *Ph. kacicola* Sawada, and 96.7% with *Leveillula taurica* (Lév.) G. Arnaud.

We compared the 28S rDNA and ITS sequences of *Ph. chubutiana* with about 100 unpublished sequences of *Phyllactinia* and *Leveillula* G. Arnaud. The result indicates that *Ph. chubutiana* has a unique DNA sequence in both 28S rDNA and ITS regions and that the fungus forms an independent lineage in the phylogenetic trees of *Phyllactinia* and *Leveillula* (unpublished data). The detailed phylogenetic analyses will be published elsewhere. These results support the morphological observation that the fungus is a distinct new species.

As mentioned earlier, the shape of conidia and the structure of conidiophores are very characteristic and different from most other *Phyllactinia* species. We thus conducted an independent sequence of the ITS region by the DNA extracted from mycelium, which was compared with the sequence from ascospores. The two sequences obtained from mycelium and ascospores are identical to one another, which confirms that the unique anamorph belongs to *Ph. chubutiana*.

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